

Clean Version of Pending Claims

SEQUENCE AND METHOD FOR INCREASING PROTEIN EXPRESSION IN CELLULAR EXPRESSION SYSTEMS

Applicant: Martin Latterich et al. Serial No.: 09/458,779

3. (Amended) A polynucleotide comprising a sequence encoding a functional vesicular fusion factor 2 protein (Vff2p), wherein the Vff2p has greater than 36% sequence identity with SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion.

- 4. (Amended) The polynucleotide of claim 3, comprising SEQ ID NO:1 or a sequence encoding SEQ ID NO:2.
- 5. The polynucleotide of claim 3, wherein the protein is about 32 kD.
- 6. The polynucleotide of claim 3, further comprising a promoter operatively linked to the sequence encoding the Vff2p.
- 7. The polynucleotide of claim 6 wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.
- 8. The polynucleotide of claim 3, further comprising a sequence encoding a heterologous target protein.
- 9. The polynucleotide of claim 8, further comprising a second promoter operably linked to the sequence encoding the target protein.

- 10. The polynucleotide of claim 9, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.
- 11. The polynucleotide of claim 7, wherein the host cell is a yeast cell.

(S

- 12. (Amended) The polynucleotide of claim 3, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.
- (3
- 14. (Amended) A polynucleotide expression vector comprising a polynucleotide encoding a functional Vff2p, wherein the Vff2p has greater than 36% sequence identity with SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion.
- 15. (Amended) The expression vector of claim 14, comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.
- 16. The expression vector of claim 14, wherein the protein is about 32 kD.
- 17. The expression vector of claim 14, further comprising a promoter sequence operatively linked to the sequence encoding the Vff2p.
- 18. The expression vector of claim 17 wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.
- 19. The expression vector of claim 14, further comprising a sequence encoding a heterologous target protein.

- 20. The expression vector of claim 19, wherein transcription of the target protein is directed by a second promoter.
- 21. The expression vector of claim 20, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.
- 22. The expression vector of claim 18, wherein the host cell is a yeast cell.
- 23. The expression vector of claim 22, wherein the yeast is Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.
- 25. (Amended) A recombinant host cell comprising a yeast cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a functional Vff2p, wherein the Vff2p has greater than 36% sequence identity with SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion.
- 26. (Amended) The host cell of claim 25, comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.
- 27. The host cell of claim 25, further comprising a sequence encoding a heterologous target protein.
- 29. (Amended) The host cell of claim 25, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell:



- 30. (Amended) The host cell of claim 25, wherein the host cell lacks a functional protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion, other than Vff2p.
- 31. (Amended) A method for increasing cell growth of a yeast host cell, comprising introducing Vff2p into the cell and culturing the cell, wherein the Vff2p has greater than 36% sequence identity to SEQ ID NO:2.
- 32. (Amended) The method for increasing cell growth of a cell according to claim 31, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.
- 33. (Amended) A method for increasing protein secretion from a yeast host cell, comprising introducing Vff2p into the cell and culturing the cell, wherein the Vff2p has greater than 36% sequence identity to SEQ ID NO:2.
- 34. (Amended) The method for increasing protein secretion from a cell according to claim 33, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.
- 36. (Amended) An isolated functional vesicular fusion factor 2 protein comprising SEQ ID NO:2, or a Vff2p with greater than 36% sequence identity to SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion.

CON't.



- 37. (Amended) A method of selecting for a yeast secretory mutant cell containing a polynucleotide sequence encoding a Vff2p operably linked to a promoter, wherein the Vff2p comprises SEQ ID NO:2, or a Vff2p with greater than 36% identity to SEQ ID NO:2, the method comprising growing the yeast secretory mutant cell at a restrictive temperature of about 32-37°C, wherein the restrictive temperature selectively favors mutant cell growth.
- 38. The method of claim 37, wherein the temperature is at about 37°C.
- 39. The method of claim 37, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, uso1-1, pex3-1, sed5-1, cdc48-2, sec7-5, or ypt1-3.28.
- 40. The method of claim 39, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, uso1-1, or pex3-1.
- 41. The method of claim 40, wherein the secretory mutant cell is sec18-1.
- 42. The method of claim 37, wherein the polynucleotide further comprises a sequence encoding a heterologous target protein operably linked to a second promoter.
- 43. The polynucleotide of claim 3, wherein the Vff2p comprises SEQ ID NO:2.
- 44. (Amended) The protein of claim 36, wherein the yeast is S. cerevisiae.
 - 45. The protein of claim 36, wherein the protein is from S. cerevisiae.

46. The method of claim 37, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell.